EFFECT OF TEMPERATURE ON BUTANOL AND ETHANOL PRODUCTION FROM PALM OIL MILL EFFLUENTS BY *CLOSTIDIUM ACETOBUTYLICUM*

EINAYAH BINTI KAMARUZAMAN

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

MEI 2010

ABSTRACT

Palm oil mill effluents is a waste that produce in high quantity in Malaysia. Furthur studies would futher increase profitability of palm oil industries besides solving environmental problems. POME has great potential as substrate because it has low cost and also it contains a mixture of carbohydrates binding, hemicelluloses, sucrose, and other carbohydrates that can be utilized by saccharolytic clostria.This research mainly to study the effect of temperature to the solventogenic fermentation using c*lostridia.acetobutylicum* in producing butanol and ethanol by using palm oil mill effluent (POME) as a fermentation media. This research also to investigate the type of sugar that contain in POME, the profile growth rate of *clostridia acetobutylicum* and then to investigate consumption of glucose by *clostridia acetobutylicum* during fermentation. The experiment works were conducted in schott bottle in anaerobic chamber to maintain anaerobic condition. Fermentation was carried out for 72 hour at 35°C with the concentration of POME at 90% and inoculums concentration at 10%. Palm oil mill effluent and reinforced clostridia medium was used as a growth medium in batch culture. The temperature that used in this investigation is 33°C, 34°C, 35°C and 37°C. The result of this investigation showed that POME is a viable media for butanol and ethanol fermentation. The result also showed that the yield of butanol and ethanol production will decrease as the temperature increase. The results showed that the highest yield of butanol produced was 0.156 g/L. For ethanol production, the highest was 58.51 g/L. The experimental results also showed that the sugar groups that contains in POME are fructose, glucose, galactose, sucrose and lactose. From this study, it was observed that optimum condition for butanol and ethanol fermentation by *Clostridia acetobutylicum* at 35°C.

ABSTRAK

Bahan buangan daripada kilang kelapa sawit atau dikenali sebagai POME, merupakan bahan terbuang yang banyak terdapat di Malaysia. Kajian yang lebih lanjut boleh meningkatkan keuntungan kepada industri kelapa sawit selain daripada menyelesaikan masalah pencemaran alam sekitar. POME mempunyai potensi yang besar sebagai media fermentasi solventogenik kerana ia mempunyai harga yang rendah dan juga mempunyai campuran ikatan karbohidrat, hemicellulose, sukros dan lain – lain karbohidrat yang boleh digunakan oleh saccharolytic clostridia. Kajian ini bertujuan untuk mengkaji kesan suhu terhadap fermentasi solventogenik menggunakan *clostridia acetobutylicum* dalam menghasilkan butanol dan ethanol menggunakan POME sebagai media juga. Kajian ini dijlalankan untuk mengkaji kandungan gula yang terdapat di dalam POME, kadar pertumbuhan *clostridia acetobutylicum* dan mengkaji kadar penggunaan gula oleh *clostridia.acetobutylicum* semasa process fermentasi. Eksperimen ini dijalankan di dalam schott botol menggunakan ‘anaerobic chamber’ untuk mewujudkan keadaan tanpa oksigen. Proses fermentasi dijalankan selama 72 jam pada 35°C dengan kepekatan POME dikekalkan pada 90% dan kepekatan inoculum pada 10%. Suhu yang digunakan dalam kajian ini ialah 33°C, 34°C, 35°C dan 37°C. Keputusan daripada kajian ini menunjukkan POME merupakan medium yang sesuai untuk fermentasi butanol dan ethanol.Hasil daripada kajian juga menunjukkan kadar penghasilan butanol dan ethanol akan menurun apabila suhu meningkat. Keputusan juga menunjukkan kadar penghasilan butanol yang tertinggi adalah pada 0.156 g/L. Untuk penghasilan ethanol pula sebanyak 58.51 g/L. Keputusan kajian juga menunjukkan terdapat beberapa kumpulan gula yang terdapat di dalam POME iaitu fruktose, glukose, sukrose, dan laktose. Daripada kajian ini, ia menunjukkan keadaan optimum untuk fermentasi butanol dan ethanol dengan menggunakan *Clostridia acetobutylicum* adalah pada 35°C.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

ABE	-	Acetone – butanol – ethanol
BOD	-	Biological oxygen demand
COD	-	Chemical oxygen demand
^{o}C	-	degree Celcius
DNS	-	Dinitrosalicylic acid
eg	-	Example
GCFID	-	Gas Chromatography(Flame Ionization Detector0
g	-	gram
HPLC	-	High Performance Liquid Chromatoghraphy
hr	-	hour
L	-	Liter
MT	-	Metric tonne
ml	-	mililiter
µm	-	micrometer
min	-	minute
POME	-	Palm Oil Mill Effluents
UV – Vis	-	UltraViolet Vision

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.0 Background of Study

Palm oil is one of the world's chief edible oils produced by South East Asian and African countries, used for producing various food products, cosmetics and pharmaceutical products and oleo chemicals. Palm oil production is one of the major industries in Malaysia. During the period between 1990 and 2002, palm oil production was nearly doubled from 6 094 622 to 1 188 000(MT) per year, making Malaysia the biggest palm oil producer worldwide. Its production generates various wastes chief among which is palm oil mill effluent (POME) (Khalil *et.al.*, 2003).

Malaysia is a world leader in palm oil industry, producing about 11.8 million tons of crude palm oil in 2001. Recycle uses of biomass generated from the production process of palm oil is an urgent subject to Malaysia, because, since the volume of palm oil mill effluent (POME) is proportional to the 3.6 times as much volume of the crude palm oil production, it gradually has become difficult to treat it only by natural evaporation at reservoirs. For this purpose, various technologies that recover useful materials and innovative organic materials from POME are being developed (Monot *et.al*, 1982). At an average, about 0.1 tonne of raw palm oil mill effluent (POME) is generated for every tonne of fresh fruit bunch processed. POME consists of water soluble components of palm fruits as well as suspended materials like palm fiber and oil. Despite its biodegradability, POME cannot be discharged without first being treated because POME is acidic and has a very high biochemical oxygen demand (BOD) (Monot *et.al.*, 1982).

Concerns about the greenhouse effect, as well as legislation to reduce CO_2 emissions and to increase the use of renewable energy have been the main reasons for the increased production and use of biofuels. In addition to bioethanol and biodiesel production, the research on biobutanol production has also increased during the past years. Butanol can be produced by chemical or biochemical routes. Fuel properties of butanol are considered to be superior to ethanol because of higher energy content, and better air-to-fuel ratio. Butanol is also less volatile and explosive than ethanol, has higher flash point and lower vapor pressure which makes it safer to handle (Pakkila *et.a.l*, 2009).

Production of industrial butanol and acetone via fermentation, using *Clostridia acetobutylicum*, started in 1916, during World War I. Chime Weizmann, a student of Louis Pasture, isolated the microbe that made acetone. England approached the young microbiologist and asked for the rights to make acetone for cordite. Up until the 1920s acetone was the product sought, but for every pound of acetone fermented, two pounds of butanol were formed. A growing automotive paint industry turned the market around, and by 1927 butanol was primary and acetone became the byproduct (Monot *et.al.*, 1982).

Butanol, which is an excellent biofuel, has numerous other applications in the food, plastics, and chemical industries (Masngut *et.al.*, 2007). Clostridia acetone/butanol fermentation used to rank second only ethanol fermentation by yeast in its scale of production and thus are one of the largest biotechnological processes known.

Butanol (butyl alcohol) is an organic compound used largely as an industrial solvent. However, when it is produced using biological materials (biomass) for feedstocks, it is called biobutanol and is no different than butanol produced using fossil fuels like oil. Since it relies on sunlight and photosynthesis to contribute to the growth of that biomass (plants, grasses, corn, wheat, etc), biobutanol is a renewable fuel (Monot *et.al.*, 1982). The production of butanol for fuel was traditionally accomplished by fermenting biomass, such as algae, corn, and other plant materials containing cellulose that could not be used for food and would otherwise go to waste.

Ethanol or ethyl alcohol (C_2H_5OH) is a clear colourless liquid; it is biodegradable, low in toxicity and causes little environmental pollution if spilt. Ethanol burns to produce carbon dioxide and water. Ethanol is a high octane fuel and has replaced lead as an octane enhancer in petrol. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces polluting emissions. Ethanol fuel blends are widely sold in the United States.

Bioethanol is one of the renewable energy source that is fast gaining foothold as potential fuel to power automotive engine. Contrary to gasoline which is refined through distilling crude fossil fuel, bioethanol can be synthesized from the starchy parts of natural plants or other biomass. Microscopic yeast cells break down the starch and water, creating the so called bioethanol and carbon dioxide as end products. Bioethanol burns to to produce carbon dioxide and water in complete combustion, a process akin to gasoline. It also possesses a high octane fuel. Bioethanol can be produced via traditional methods such as fermentation, and it can be distributed using the same petrol forecourts and transportation systems as before. In the biomass to bioethanol process, acids and enzymes are used to catalyze the reactions. (Cheng *et.al.*, 2007).

The fermentation process is facilitated mainly by a type of bacteria called *C. acetobutylicum*. Oddly enough, these bacteria are rather closely related to those which cause botulism. Other microorganisms are also able to ferment these materials, and research into these types of production techniques is ongoing (Ishizaki *et.al.*,1999).

The acetone – butanol – ethanol (ABE) fermentation was the largest scale bioindustry ever run second second to ethanol fermentation (Antoni *et.al.*, 2007). Acetone – butanol fermentation usually represents the first stage of growth and acid production followed by the second stage where solvents are produced with partial reutilization of the acids (Lepage *et.al.*, 1987). However, the traditional clostridial fermentation of butanol and acetone is suffering from the difficulties of switching the acidogenic fermentation stages to the solventogenic stage, and thus, a discontinuous production mode, from common phage infections, the rising substrate costs and the effort required for downstream processing (Antoni *et.al.*, 2007). In 1927 butanol became the main product of ABE process. In the time of Second World War it was used for producing synthetic rubber. In the first half of 20th century biobutanol was produced from corn and molasses by fermentation. This process affords acetone, butanol and ethanol (therefore it is denoted as ABE process). However with growing demand for butanol its producing by fermentation began to fall because since 1954 the price of petroleum becomes below those of sugar because USA lost cheap sugar supply from Cuba. Now butanol is produced starting with petroleum via hydrolysis of haloalkanes or hydration of alkenes (Shapovalov *et.al.*, 2008).

1.1 Problem Statement

Concerns about the greenhouse effect, as well as legislation to reduce CO_2 emissions and to increase the use of renewable energy have been the main reasons for the increased production and use of biofuels. Although the butanol price is higher than the ethanol, but production of butanol comes with many advantages. Also, traditional fuel pipelines cannot be used with ethanol since water mixes into it, but butanol does this to a lesser extent and so could be used with more existing infrastructure.

Palm oil mill effluents are one of the most abundant wastes in Malaysia. They are mainly organic in nature and are highly polluting. The biological oxygen demand and chemical oxygen demand (BOD and COD) of this effluent is very high and also acidic. Thus, if such an effluent with its quality and quantity were to be discharged into the rivers, all the aquatic life will perish. POME has great potential as substrate because is has low cost and also it contains a mixture of carbohydrates binding starch, hemicelluloses,sucrose, and other carbohydrates that can be utilized by saccharolytic clostria. Such utilization would futher increase profitability of palm oil industries besides solving environmental problems.

Petroleum resources are the only major mineral commodities where many experts fear resource depletion will produce significant scarcities over the next several decades. By producing solvents such a ethanol or butanol, it can overcome this problems. Progress in the area of biotechnology allows using corn and other biomass as economically effective source of biobutanol. The alcohol based fuel including butanol and ethanol is partially oxidized (compared to hydrocarbons) and therefore mixture for engine should be more enriched than in the case of gasoline.

1.3 Objective of Study

To study the effects of temperature (33°C to 37°C) on solvent production (butanol and ethanol) from palm oil mill effluent (POME) by *Clostridium acetobutylicum*.

1.4 Scope of Study

1. To study the growth profile of *Clostridia acetobutylicum* in POME and (Reinforced Clostridia Medium).
2. To analyze the composition of POME using High Performance Liquid Chromatography (HPLC).
3. To study the effect of temperature (33°C to 37°C) to the solvents productions using POME.
4. To study glucose consumption in the fermentation broth.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Mill Effluents (POME)

The palm oil industry, apart from being a major foreign exchange earner for Malaysia,is also identified as the single largest source of water pollution. It produces a large volume of highly polluting effluents, for instance, 2.5 tonnes of Palm Oil Mill Effluent (POME) is generated for every tonne of crude palm oil produced (Abdullah *et.al*,2004).The improvement of processing technology will lead to further increase in world's palm oil supply. However, the rapid development of the industry has had serious consequences on the natural environment, which mainly related to water pollution due to a large discharge of untreated or partially treated palm oil mill effluent (POME) into watercourses. In the year 2004, more than 40 million tonnes of POME was generated from 372 mills in Malaysia (Abu *et.al.*, 2007).

POME is high volume liquid wastes which are non toxic but have an unpleasant odour. They are predominantly organic in nature and are highly polluting. The biological oxyegen demand and chemical oxygen demand (BOD & COD) of this effluent is very high and so goes for the total nitrogen, ammonical nitrogen and oil and grease. The effluent is also acidic. Other than this the raw effluent is made up of a few anions and cations. As for the physical nature of the raw effluents, it is hot, has a bad aroma and is brown in color.

Thus, if such an effluent with its quality and quantity were to be discharged into our aquatic life will perish (Khatiravale *et.al.*, 1997).

POME is produced from production of crude oil which involves extraction process where the fresh palm oil friuit bunches undergo sterilization, digestion and extraction of the oil, which is then clarified. POME is produced in vast amounts through the year.palm oil mill effluents has great potential as a substrate for ABE fermentation because it contains a mixture of carbohydrates including starch, hemicellulose, sucrose and other carbohydrates that can be utilized by saccharolytic clostridia (Khalil *et.al.*,2003).

Fresh POME is a colloidal suspension containing about 95% water, 0.6-0.7% oil, and 4-5% total solids including 2-4% suspended solids that are mainly debris from palm fruit (Ahmad *et al.*, 2005). It is acidic with pH 4-5 and discharged at temperature about 80-90°C. Although the effluent is non toxic, it has a very high concentration of biochemical oxygen demand (BOD) (25 000 mg/L) which becomes a serious threat to aquatic life when discharged in relatively large quantities into watercourses. Furthermore, POME contributes 83% of the industrial organic pollution load in Malaysia (Abu *et.al.*,2007).Palm oil mill effluents (POME) has great potential as a substrate for ABE fermentation because it contains a mixture of carbohydrates including starch, hemicelluloses, sucrose and other carbohydrates that can be utilized by saccarolytic clostridia(Khalil *et.a.l*,2003).

2.2 Solventogenic Clostridia

The solventogenic clostridia have received much attention in recent years, because of their ability to produce industrially relevant chemicals such as butanol and 1, 3-propanediol. The clostridia secrete numerous enzymes that facilitate the breakdown of polymeric carbohydrates into monomers (Ezeji,T.C *et.al.*, 2007). Clostridia have a long history of being employed in several biotechnological processes, for instance, *C.*

acetobutylicum in the conversion of renewable biomass for acetone/butanol production (Patova *et.al.*, 2009).

C. acetobutylicum is an anaerobic, zeterofermentative organism. It also is able to use polymeric substrates such as starch and xylan, but not cellulose, for growth (Durre, 1998). Anaerobic bacteria such as the solventogenic clostridia are capable of converting a wide range of carbon sources (e.g.glucose, galactose, cellobiose, mannose, xylose and arabinose) to fuels and chemicals such as butanol, acetone, and ethanol (Ezeji *et.al.*, 2007) (Masngut *et.al.*, 2007).

Studies on the production of solvents have always been done on natural media; yet, it is well known that *C. acetobutylicum* can grow on a sugar-salt-vitamin medium (Masngut *et.al.*, 2007). Several species of Clostridium bacteria are capable to metabolize different sugars, amino and organic acids, polyalcohols and other organic compounds to butanol and other solvents (Nik *et.al.*, 2004).

The growth of *C. acetobutylicum* utilizing both carbon sources can be divided into two phases, acidogenic phase and solventogenic phase. An acidogenic phase where organic acid (acetic and butyric acid) were actively produced which cause the reduction in culture pH. At the same time glucose is also actively consumed to accommodate the high growth rate in the culture (Liew *et.al.*, 2006).

In recent studies, the intracellular pH of *C. acetobutylicum* cells grown in a chemostat decreased with decreasing external pH during acetogenic fermentation and then become stabilized after solventogenesis was initiated (Huang *et.al.*,1986).

In clostridia fermentation, the sporulation occurs concomitantly with the solventogenesis. Sporulation makes the bacterial cells enter a dormant state where they lose the ability to produce solvents. It is likely that there is a relationship between sporulation and solventogenesis, given that many early molecular events connected with sporulation appear in the initiation of solventogenesis.If this relationship is revealed, it may be possible to produce more solvents, including butanol, by preventing the clostridia from forming spores. (Wu *et.al.*, 2003).

During fermentation, *C. acetobutylicum* produces three major classes of products; solvents (acetone, ethanol and butanol); organic acids (acetic acid, lactic acid and butyric acid); gases (carbon dioxide, and hydrogen) (Wu *et.al.*, 2003).

In batch culture exponentially growing cells produce organic acids which lower the pH of the medium. As the culture enters the stationary phase the metabolism of the organism changes: carbohydrate and the preformed organic acids are converted into organic solvents (Bowles *et.al.*, 1985).

The biobutanol fermentation suffers from several limitations (e.g. low titer, yield and productivity) and improvements in the performance of the solventogenic clostridia are necessary to move biobutanol fermentation research to a competitive commercial position. Several approaches have been employed to improve the performance of solventogenic clostridia with the aim of generating strains that can be used in industrial biobutanol production. Recombinant DNA technology, in addition to traditional mutagenesis and selection, have been employed to modify targeted metabolic pathways in the solventogenic clostridia (Ezeji *et.al.*, 2007).

An important advantage of the solventogenic clostridia is the variety of fermentation products (acetone, butanol, ethanol, acetic, butyric, lactic acids, etc.) that can be synthesized by this group of microorganisms. However, the loss of available carbon as a result of the formation of unwanted products is an undesirable property of the solventogenic clostridia. Clearly, enzyme synthesis and control of electron flow in the glycolytic pathway are vital with respect to the regulation of the butanol fermentation pathways. (Ezeji *et.al.*, 2007).

In addition, the ability of the solventogenic clostridia to grow under a low redox potential enables them to undertake a variety of stereospecific reductions, yielding chiral products that are difficult to synthesize chemically (Bowles *et.al*, 1985). As the electron flow can be reversed, butanol yield should respond to factors that influence the direction of electron flow (Ezeji *et.al.*, 2007).

2.3 Fermentation

2.3.1 Anaerobic Fermentation

Anaerobic fermentation is the process of fermentation without using any oxygen. One of advantages of the anaerobic process is the recovery of the useful matters such as solvents (Hwang *et.al.* 2004).

The most important economic factor in solvent fermentation is the cost of substrate, which made up about 60% of the overall cost of production. (Liew *et.al.*,2006). Biobutanol production is an anaerobic two-stage fermentation process where acetic and butyric acids, carbon dioxide and hydrogen are first produced in the acidogenic phase. Then the culture undergoes metabolic shift to solventogenic phase and acids are converted into acetone, ethanol and butanol. At the end of the fermentation, products are recovered from the cell mass, other suspended solids, and by-products (Pakkila *et.al.*, 2009).

2.3.2 ABE Fermentation

The ABE fermentation was the largest scale bioindustry ever second run to ethanol fermentation. (Khalil *et.al.*, 2003) Its decline since about 1950 has been caused by increasing substrate costs and the availability of much cheaper feedstock for chemical solvent synthesis by the petrochemical industry (Durre., 1998).

In particular, biobutanol results a good candidate as transportation fuel and ABE fermentation is a potential path to upgrade biomass into valuable liquid fuels (Napoli *et.al.*, 2009).

Acetone-butanol fermentation by *C. acetobutylicum* has been characterized as biphasic batch-culture fermentation (Huang *et.al.*, 1986) (Kanouni *et.al.*, 1998). The first phase is characterized by rapid growth and by the formation of acetic and butyric acids which are excreted into the medium, thereby lowering the medium pH. The second phase commences after the pH of the medium has fallen below approximately 5.0. During this period, butanol and acetone become the major fermentation products. The fatty acids, previously accumulated in the medium, pass through the cell membrane in their undissociated form and are converted to solvents. At the end of the fermentation, the metabolic activity ceases primarily because the concentrations of the solvents have reached toxic levels (Huang *et.al.*, 1986).

The major end product of the fermentation is butanol, with acetone and ethanol being minor products (Bowles *et.al.*, 1985). All the butanol that have been produced along with acetone was considered a by – product and kept in large storage tanks (Durre., 1998). The production of the byproducts could be reduced or terminated, and the process could greatly benefit from new and cheaper substrates derived by hydrolysis from lignocelluloses biomass, as well as from advanced sterile fermentation and downstream possessing technology (Khalil *et.al.*, 2003).

Batch fermentation by *C. acetobutylicum* is characterized by two phases. During the first phase, or acidogenesis, *C. acetobutylicum* grows and produces acetate and butyrate from glucose. These acids attain their maximal concentrations and are consumed in the second phase, which is known as solventogenesis. The acids are reduced, and neutral solvents including butanol, acetone, ethanol, and acetone are produced (Evans *et.al.*, 1988).

2.4 Butanol

First of all, butanol is used as industrial solvent.World market for this product is estimately 350 million gallons per year, of which 220 million gallon/year is the fraction consumed by USA. Butanol can be used instead of gasoline even in higher degree than ethanol due to its physical properties, economy, safety and because it can be applied without remodeling car engine. (Shapovalov *et.al.*, 2008).

By 1927 butanol was increasingly used for the production of the lacquer solvent butyl acetate and for the synthetic rubber industry. Japan, and other possible combatants, used butanol as an aviation fuel during WWII when they exhausted their fossil fuel supply (Khalil *et.al.*, 2003).

The factors which severely affect the economics of butanol fermentation were high cost of substrate; low product concentration, low reactor productivity, low ABE yield, and escalated cost of butanol recovery by distillation which was the only techniques for recovery at old time. The cost of recovery of butanol is high due to the fact that its concentration in the fermentation broth is low because of the product inhibition (Qureshi *et.al.*, 2001)

Today, new uses for butanol are emerging, as a diesel and kerosene replacement, as silage preserver, biocide and C4 compound for chemical industries (Khalil *et.al*, 2003).

Butanol, which is an excellent biofuels, has numerous other applications in the food, plastics, and chemical industries (Ezeji *et.al.*, 2005).

The main reason that nobody knows butanol as an alternative fuel is the fact that its producing has never been suggested economically reasonable. As mentioned above, this product is used mainly as industrial solvent and costs triple compared to gas (Shapovalov *et.al.*, 2008). Butanol has a heat of combustion sufficiently high to allow for their use in high thrust – to – weight applications such as airplanes. For safe and environmentally friendly storage, vapor pressure and ignition temperature are important factors (Khalil *et.al.*, 2003).

Starch and starch based co – products can be used for conversions to fuels and chemicals such as butanol. The production of amylolytic enzymes (α – amylases and amyloglucosidases) by the production microorganism, known as saccharification fermentation, has an advantage over systems where hydrolysis and subsequent fermentation.(Ezeji *et.al,* 2005). Butanol is generally produced in concentrations of no greater than 12 g/liter .This limitation is thought to be due to the toxicity of butanol to *C.acetobutylicum* (Evans *et.al*, 1988).

Butanol toxicity to the fermenting microorganisms limits its concentration in the fermentation broth, resulting in low butanol yields and a high cost for butanol recovery from the dilute solutions.During the past decade, the application of molecular techniques to the solventogenic clostridia—combined with recent advances in fermentation techniques — have resulted in the development of a hyper-butanol-producing strain and an integrated ABE fermentation system for the simultaneous production and removal of butanol from the fermentation broth (Ezeji *et.al.*, 2007). One technique for increasing butanol production is genetic alteration of *C. acetobutylicum* to make it more tolerant to butanol (Evans *et.al.*, 1988).

2.5 Ethanol

Ethanol, with its high (C+H) to O ratio, retains most of the original energy content in combustion. Ethanol does not need to be rectified to high purity if it is to be used as a fuel (Bowles *et.al.*, 1985). Ethanol also called ethyl alcohol, pure alcohol, grain alcohol, or drinking alcohol is a volatile, flammable, colorless liquid. It is a powerful psychoactive drug, best known as the type of alcohol found in alcoholic beverages and in modern thermometers.

Ethanol is one of the oldest recreational drugs. In common usage, it is often referred to simply as alcohol or spirits. Ethanol, a traditional biofuel, is not an ideal fuel due to its high hygroscopic and low energy density, which increased the difficulty involved in and expense of its storage and distribution (Wu *et.al.*, 2003).

The fermentation if sugar into ethanol is one of the earliest organic reactions employed by humanity. The intoxicating effects of ethanol consumption have been known since ancient time. In modern times, ethanol intended for industrial use is also produced from byproducts of petroleum refining. Ethanol is a volatile, colorless liquid that has a strong characteristics odor. It burns with smokeless blue flames that are not always visible in normal light.

2.6 Advantages of butanol from ethanol

The alcohol based fuel including butanol and ethanol is partially oxidized (compared to hydrocarbons) and therefore mixture for engine should be more enriched than in the case of gasoline. As compared with ethanol, butanol can be used as a mixure with gasoline in higher proportion and thus can be used in currently working cars without modifi cation of their system for the formation of air–fuel mixture. The alcohol based fuel contains less energy per unit of weight or volume than gasoline and its mixture with air should be more enriched. Per one cycle of engine running, butanol liberates more pure energy than ethanol (Hwang *et.al*, 2004) (R.Gheshlaghi *et.al*, 2009).

There are several advantages of butanol over ethanol:butanol contains 25% more energy than erthanol : 110 000 Britain heat units per gallons as compared with 84 000. Britain heat units per one gallons of ethanol. Energy content of gasoline is about 115 000 Britain heat units per gallons. Butanol is safer because is evaporated six times less than ethanol and by factor 13.5 less volatile than gasoline. Its vapor pressure by Reid is 0.33 pounds per squire inh,the same characteristic of gasoline is 4.5 pounds per square inch, of ethanol 2.0 pounds per square inch. This makes butanol safe at its application as oxygenate, and need no significant changes in the mixture proportion at summer and winter application. Butanol is much less aggressive substances than ethanol and therefore it can be transported with the currently used fuel pipes, while ethanol should be transported by rail way transport (Hwang *et.al*, 2004).

Butanol also can be mixed with gasoline. Butanol can be used instead of gasoline, while ethanol can be used as additive to benzene with content of the latter in the mixture not less than 85% and requires significant modernization of engine. Currently, predominantly are used mixtures with content of ethanol 10%. Production of butanol can simultaneously solve problems that connected with the infrastructures of supplying hydrogen. Besides that, butanol provides higher yield (10Wt-h/g) than ethanol (8Wt-h/g). At combustion, butanol does not produce sulfur and nitrogen oxides that are advantages from the ecology viewpoint.

Thus, biobutanol allows solving a part of problems retarding the fast application of biofuel over the world, in part; it is compatible with existing car fuel and with infrastructure of logistics. Low vapor pressure of biobutanol and its low sensitivity to the presence of water in its mixture with gasoline extend its versatility. It can be mixed with gasoline to higher concentration than existing biofuels without modernization of engines, economically it is preferred than mixture of ethanol with gasoline, it improves efficiency of cars in respects of fuels and increases run per a unit of consumed fuel.

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Strain

The solvent – producing – bacterium, *C.acetobutylicum* (NCIMB 13357) was used throughout this study. This strain was obtained from Universiti Kebangsaan Malaysia (UKM). The culture was kept in the freezer.

To strike the bacteria on Petri dish, the anaerobic chamber was used to maintain anaerobic condition. Then from the pure stock *C acetobutylicum* was inoculated to the plate in three parallel lines about 5mm distance from each others. After striking the bacteria on Petri dish, it was sealed parafilm. Then it will incubated in the incubator at 37°C for 2 days.